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Effects of breed and sex on platelet and white blood cell counts in equine platelet-rich plasma and peripheral blood

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ABSTRACT

Platelet-rich plasma (PRP) is increasingly used in equine medicine, but its composition and therapeutic efficacy vary depending on preparation methods and intrinsic factors such as breed and sex. To evaluate the effects of breed and sex on platelet (PLT) and white blood cell (WBC) counts in peripheral blood and PRP of Thoroughbred (TB) and Purebred Arabian (PA) horses, forty-one clinically healthy racehorses (23 TB, 18 PA; aged 3–6 years) were sampled. PRP was prepared using a three-step centrifugation protocol. PLT and WBC counts were measured with an automated hematology analyzer and confirmed microscopically. Data were analyzed using t-tests or Mann–Whitney U tests ($p < 0.05$). Peripheral WBC counts were significantly higher in TB than PA horses (8.79 ± 1.28 vs. 7.60 ± 1.67 $\times 10^3/\mu L$; $p = 0.013$), while peripheral PLT counts did not differ by breed ($p = 0.269$). PRP PLT counts were significantly higher in TB compared to PA horses (845.77 ± 316.34 vs. 647.00 ± 213.84 $\times 10^3/\mu L$; $p = 0.033$). Regarding sex, no significant differences were found in peripheral WBC ($p = 0.720$) or PLT counts ($p = 0.423$). In PRP, WBC counts were significantly higher in males compared to females (52.97 ± 29.72 vs. 40.37 ± 20.05/µL; $p = 0.039$), while PRP PLT counts showed no sex-related difference ($p = 0.445$). Both breed and sex influence the cellular composition of equine PRP. Thoroughbreds yield higher PRP platelet concentrations than Arabians, and males exhibit higher PRP leukocyte levels than females. These intrinsic factors should be considered when interpreting hematological data and optimizing PRP-based regenerative therapies. Future studies with larger, multi-breed cohorts are warranted to refine standardization efforts.

Keywords: regenerative therapy, purebred Arabian horse, thoroughbred horse, equine hematology

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Introduction

Platelet-Rich Plasma (PRP) is a concentrated suspension of platelets derived from whole blood and is increasingly used in both human and veterinary medicine for its regenerative potential. PRP has become a popular biologic therapy among equine practitioners (Brossi et al., 2015; Knott et al., 2022) , with studies reporting its use in conditions such as tendon and ligament injuries, joint disorders, and osteochondral lesions (Brossi et al., 2015; Castelijns et al., 2011; Seabaugh et al., 2017). Nevertheless, despite its widespread use, clinical efficacy remains inconsistent across studies, likely due to variation in both preparation methods and patient-related factors (Peng et al., 2024; Rinnovati et al., 2016)

The therapeutic effects of PRP are largely attributed to platelets, which release growth factors and cytokines that stimulate angiogenesis, tissue regeneration, and the recruitment of inflammatory cells essential for

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wound healing (Satué et al., 2017; Soares et al., 2021). However, PRP is not solely composed of platelets. Leukocytes are also present in varying amounts depending on the preparation method, and they play a crucial role in modulating inflammation and enhancing tissue repair. Their contribution can significantly affect treatment outcomes; for example, leukocyte-rich PRP (L-PRP) has been shown to promote fibroblast activation and matrix metabolism more effectively than leukocyte-poor PRP (LP-PRP) (Devereaux et al., 2020; Yin et al., 2016).

Because both platelet concentration and leukocyte content directly influence the biological activity of PRP, assessing these cellular components are essential. Yet, preparation protocols differ widely—from commercial kits to manual methods—resulting in inconsistencies in platelet enrichment and leukocyte composition, and ultimately in variable therapeutic efficacy (Anitua et al., 2017; Chahla et al., 2017; Lansdown & Fortier, 2017). Another important aspect to consider is the relationship between platelet concentration in PRP and therapeutic efficacy. Contrary to the common assumption that higher platelet counts automatically result in superior outcomes, this relationship is complex and often non-linear. Various studies indicate that the effectiveness of PRP can be enhanced by specific components, such as growth factors and leukocytes, making it essential to consider an optimal concentration level (Castillo et al., 2011). The release of growth factors and clinical outcomes depend on various factors, including the specific composition of the PRP, reinforcing the notion that excessive platelet concentration does not inherently equate to enhanced therapeutic effect (Li et al., 2013; Lovering et al., 2009). Thus, it is crucial to employ methodical PRP preparation and maintain a nuanced understanding of composition effects on therapeutic outcomes (Huang & Wang, 2012).

In addition to preparation-related variables, recentlLiterature indicates that intrinsic factors including age, sex, and breed significantly influence baseline platelet levels and the composition of platelet-rich plasma (PRP) in horses. Notably, differences in platelet concentrations have been reported among various breeds, with specific studies investigating Thoroughbreds, Brazilian Criollo Horses, Brazilian Sport Horses, Miniature Horses, and Crossbred Horses. These studies demonstrate that platelet counts and PRP characteristics can vary with age and gender; younger horses often exhibit higher platelet levels compared to older ones, while female horses can show differing platelet concentrations than males (Giraldo et al., 2013; Paz et al., 2022). Specific breed-related distinctions were highlighted in several studies. For example, Paz et al. (2022) emphasized the influence of breed on platelet counts and PRP characteristics among the aforementioned horse breeds, illustrating that breed-specific factors contribute significantly to PRP variability. Moreover, Miranda et al. (2018) corroborated that breed and sex drive differential PRP quality, further underscoring the necessity to standardize PRP preparation protocols to account for such variations.

Despite these valuable insights, there remains limited data on how such intrinsic factors impact the cellular composition of PRP in racehorses. Therefore, the aim of this study was to evaluate the effects of breed and sex on platelet and WBC counts from both peripheral blood and PRP samples collected from clinically healthy TB and PA horses.

Materials and Methods

Ethical approval: This study was conducted in accordance with institutional and national guidelines for the ethical use of animals in research. The study protocol was reviewed by the Muğla Sıtkı Koçman University Experimental Animals Research and Application Center Animal Experiments Local Ethics Committee (MUDEM-HADYEK), which decided that formal ethical approval was not required (Decision No: 42/21, Meeting date: 30.11.2021). The horses enrolled in the study were presented for routine performance evaluations at the racetrack, and no procedures beyond standard clinical care were performed. During routine blood sampling, an additional volume of blood was collected for research purposes with the consent of the attending trainer or responsible individual.

Horses: A total of 41 clinically healthy racehorses were enrolled in this study, including 23 Thoroughbreds (TB) and 18 Purebred Arabians (PA), all of which were registered in the national Studbook maintained by the Ministry of Agriculture and Forestry of the Republic of Turkey. The horses were in active training at the Istanbul racecourse operated by the Turkish Jockey Club.

The horses were aged between 3 and 6 years, with a mean age of 4.17 ± 1.30 years. Of the 23 TB horses, 15 were males and 8 females; of the 18 PA horses, 11 were males and 7 females. All animals were housed in individual stalls, received a standardized feeding regime consisting of commercial grain concentrate and hay, and had free access to water.

Inclusion criteria were the absence of clinical or hematological abnormalities based on routine physical examination and complete blood count (CBC) results (Castillo et al., 2011; Giraldo et al., 2013). For each horse, venous blood was collected aseptically from the jugular vein using a 21G blood collection cannula. Samples for PRP preparation were drawn into 8.5 mL acid citrate dextrose (ACD) anticoagulant tubes, while additional samples for hematological analysis were collected into ethylenediaminetetraacetic acid (EDTA) tubes (Brossi et al., 2015; Lee et al., 2018; Paz et al., 2022).

PRP preparation: For each horse, approximately 15 mL of whole blood was collected by jugular venipuncture into two 8.5 mL ACD tubes for PRP preparation. PRP was prepared using a three-step centrifugation protocol, modified from Carmona et al. (2013). The procedure was as follows:

First centrifugation was performed at 2,400 rpm (~967 g) for 5 minutes to separate the plasma and buffy coat from red blood cells. The upper plasma fraction, including the buffy coat (approximately 8 mL), was carefully aspirated.

Second centrifugation was conducted at 3,200 rpm (~1,717 g) for 10 minutes. After centrifugation, the supernatant was partially removed, leaving approximately 4 mL of plasma containing the platelet-rich layer.

Third centrifugation was again performed at 3,200 rpm (~1,717 g) for 10 minutes. Following this step, the upper portion of the plasma was discarded, and the remaining ~2 mL of concentrated PRP was collected for analysis.

Centrifugation was performed using a benchtop centrifuge (Eppendorf 5702 R centrifuge; Eppendorf AG, Hamburg, Germany). Subsequent steps involving aspiration and transfer of plasma fractions were carried out under sterile conditions within a laminar flow biosafety cabinet. The entire process was completed within one hour of blood collection. No platelet activation agents were used during preparation.

Hematological analysis: Platelet and WBC counts were obtained before and after PRP preparation using an automated veterinary hematology analyzer (Sysmex XN -1000 Vet; Sysmex Corporation, Kobe, Japan).

To confirm analyzer results, manual estimates were performed from stained blood smears. For platelet estimation, smears were stained with a Romanowsky-type stain (May–Grünwald–Giemsa) and examined under 100× oil immersion. Platelet counts were estimated by multiplying the average number of platelets per field in 10 fields by 15,000, as described by Stirn & Freeman (2022).

For WBC counts, smears were examined at 100× magnification, and the total WBC count (cells/µL) was estimated by multiplying the average number of leukocytes observed in 10 fields by 100–150. WBC differential counts were obtained either by overall slide review or by identifying 100–200 consecutive leukocytes at 400× or 500× magnification (Stirn & Freeman, 2022).

The platelet enrichment ratio was calculated as the percentage increase in platelet concentration in PRP compared with baseline whole blood values. Samples with visible hemolysis or clot formation were excluded.

Statistical analysis: All statistical analyses were performed using SPSS software (version 26.0). The normality of data distribution was assessed using the Shapiro-Wilk test. For normally distributed variables, Independent Samples t-tests were used to compare means. For non-normally distributed variables, comparisons were made using the Mann-Whitney U test. Statistical significance was defined as $p < 0.05$.

In breed comparisons of peripheral blood parameters, only PLT values were normally distributed and thus analyzed using t-tests, while non-parametric methods were applied to other variables. When analyzing the effect of sex, WBC and PLT values from peripheral blood were normally distributed and compared using t-tests. In all visual representations, bar charts were used for normally distributed data (mean ± standard error), and box plots (median, interquartile range) were used for non-normal data.

Results

Peripheral blood parameters: The peripheral WBC count was significantly higher in TB horses (n=22; 8.79 ± 1.28 ×10^3/µL) compared to PA horses (n=18; 7.60 ± 1.67 ×10^3/µL; $p = 0.013$) (Figure 1).

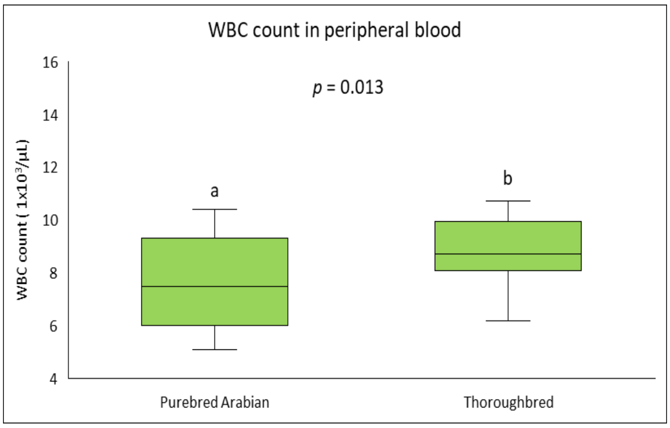

Figure 1. Effect of breed on WBC count in peripheral blood. Box plots show median and interquartile range (IQR); whiskers indicate minimum and maximum values. Different superscript letters indicate significant differences between groups ($p < 0.05$).

Peripheral PLT counts did not differ significantly between TB (n=23; 191.70 ± 61.06 ×10^3/µL) and PA horses (n=18; 171.89 ± 48.87 ×10^3/µL; $p = 0.269$) (Figure 2).

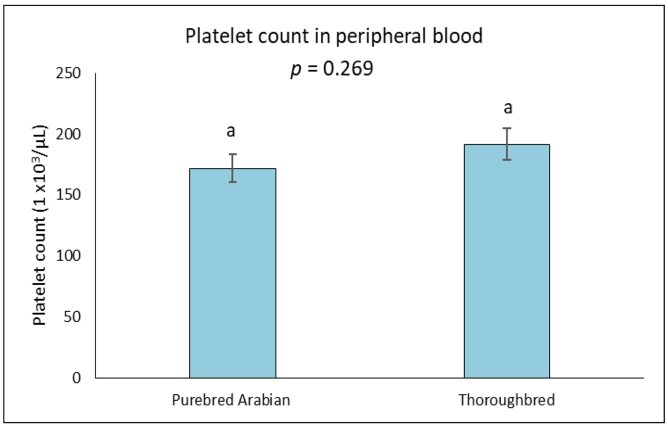

Figure 2. Effect of breed on platelet count in peripheral blood. Bars represent mean ± SE. Different superscript letters indicate significant differences between groups ($p < 0.05$).

With respect to sex, there was no significant difference in WBC counts between males (n=26; 8.32 ± 1.63 $\times10^3$/µL) and females (n=15; 8.53 ± 2.12 $\times10^3$/µL; p = 0.720) (Figure 3).

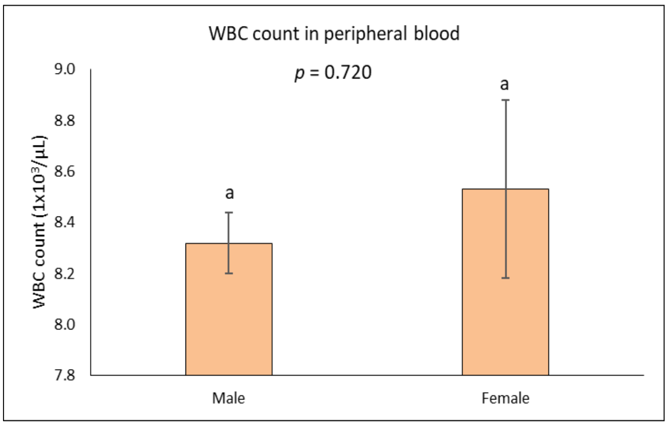

Figure 3. Effect of sex on WBC count in peripheral blood. Box plots show median and interquartile range (IQR); whiskers indicate minimum and maximum values. Different superscript letters indicate significant differences between groups ($p < 0.05$).

Similarly, PLT counts were comparable between males (n=26; 188.42 ± 45.00 $\times10^3$/µL) and females (n=15; 173.60 ± 72.64 $\times10^3$/µL; p = 0.423) (Figure 4).

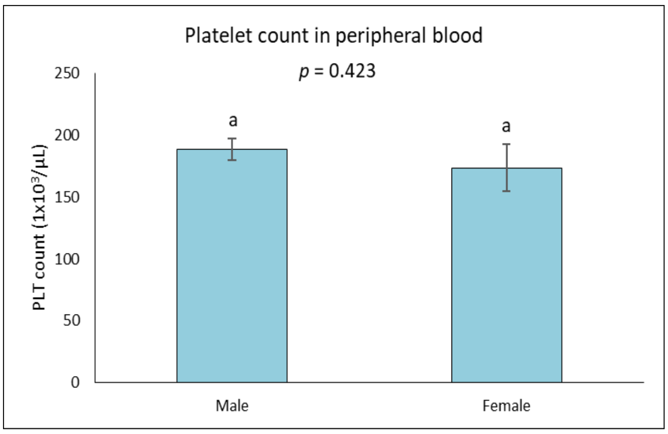

Figure 4. Effect of sex on platelet count in peripheral blood. Bars represent mean ± SE. Different superscript letters indicate significant differences between groups ($p < 0.05$).

Platelet-rich plasma parameters: In the PRP samples, WBC counts did not differ significantly between TB (n=23; 46.20 ± 25.01/µL) and PA horses (n=18; 51.12 ± 29.92/µL; p = 0.696) (Figure 5).

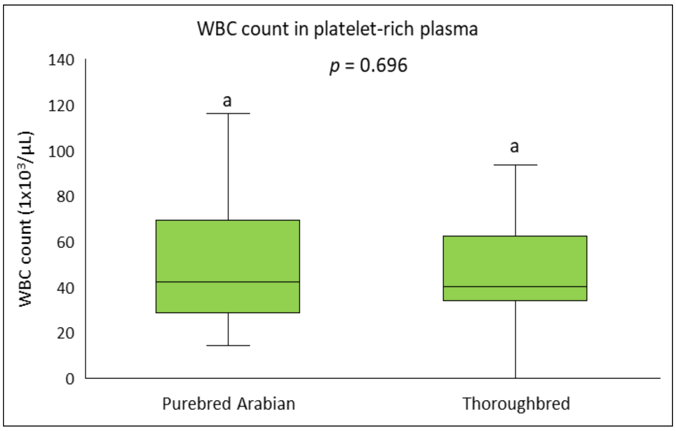

Figure 5. Effect of breed on WBC count in platelet-rich plasma. Box plots show median and interquartile range (IQR); whiskers indicate minimum and maximum values. Different superscript letters indicate significant differences between groups ($p < 0.05$).

By contrast, PRP PLT counts were significantly higher in TB horses (n=22; 845.77 ± 316.34 $\times10^3$/µL) compared to PA horses (n=15; 647.00 ± 213.84 $\times10^3$/µL; p = 0.033) (Figure 6).

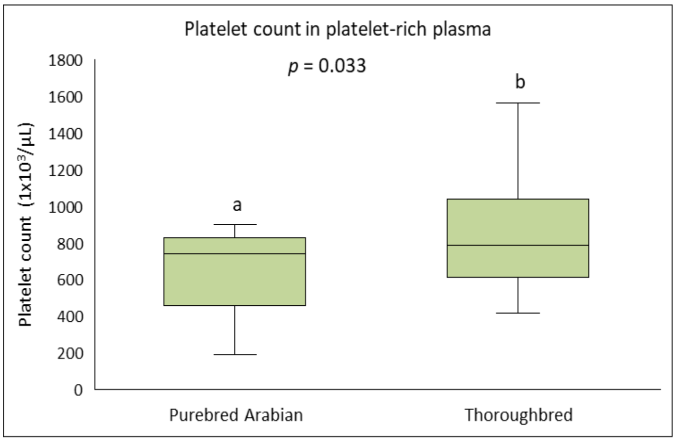

Figure 6. Effect of breed on platelet count in platelet-rich plasma. Box plots show median and interquartile range (IQR); whiskers indicate minimum and maximum values. Different superscript letters indicate significant differences between groups ($p < 0.05$).

With respect to sex, PRP WBC counts were significantly higher in males (n=26; 52.97 ± 29.72/µL) compared to females (n=15; 40.37 ± 20.05/µL; p = 0.039) (Figure 7).

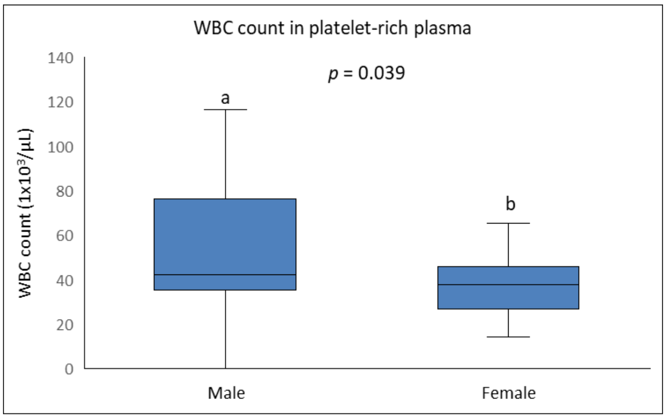

Figure 7. Effect of sex on WBC count in platelet-rich plasma. Box plots show median and interquartile range (IQR); whiskers indicate minimum and maximum values. Different superscript letters indicate significant differences between groups ($p < 0.05$).

No significant difference in PRP PLT counts was observed between males (n=26; 941.23 ± 488.67 $\times 10^3/\mu L$) and females (n=15; 763.60 ± 342.56 $\times 10^3/\mu L$; p = 0.445) (Figure 8).

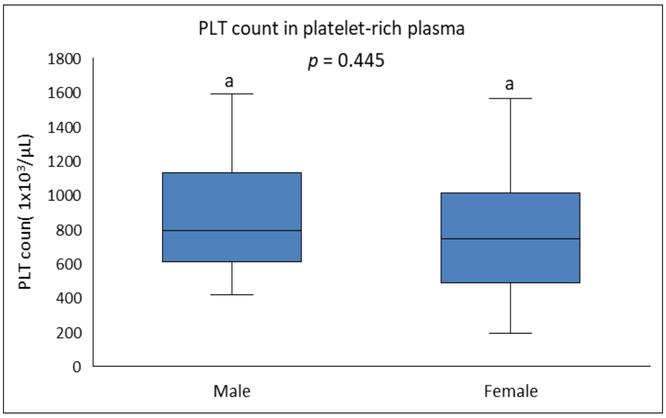

Figure 8. Effect of sex on platelet count in platelet-rich plasma.
Box plots show median and interquartile range (IQR); whiskers indicate minimum and maximum values. Different superscript letters indicate significant differences between groups ($p < 0.05$).

Discussion

This study investigated the effects of breed and sex on WBC and platelet counts in both peripheral blood and PRP of clinically healthy Thoroughbred and Purebred Arabian horses. Our findings confirm that intrinsic factors can influence hematological characteristics relevant to PRP preparation.

Breed-related effects on WBC and PRP platelets: We found significantly higher peripheral WBC counts in TB compared to PA horses. Breed-related differences in WBC dynamics have been previously described, reflecting genetic variability and physiological adaptation to exercise and training regimens (de Siqueira et al., 2019; Grizendi et al., 2020) . Environmental factors, such as management conditions, also contribute to leukocyte variation across breeds (Mahrous et al., 2011) . Interestingly, some studies suggest PA horses may exhibit higher baseline WBC counts than TB horses (de Siqueira et al., 2019) , which underlines the complexity of comparing different equine populations and supports the need for standardized reference ranges.

Beyond peripheral blood, our results demonstrated that TBs produced significantly higher platelet concentrations in PRP than PA horses. This is consistent with evidence showing breed-dependent variability in platelet recovery and PRP quality (Giraldo et al., 2013; Paz et al., 2022). Differences may be due to baseline platelet levels, hematological characteristics, or responsiveness to centrifugation protocols. In addition, centrifugation settings themselves play a critical role in PRP yield, with higher g-forces and optimized spin times increasing platelet recovery (Barros Pedroso et al., 2021). As suggested in recent work, breed-tailored protocols may therefore help optimize PRP efficacy in equine practice (Dawod et al., 2021).

Sex-related effects on PRP WBC and platelets: We observed significantly higher WBC counts in PRP samples from male horses, a difference not present in peripheral blood. While Miranda et al. (2018) did not report consistent sex effects, other equine and human studies highlight sex-dependent differences in WBC subpopulations, possibly mediated by hormonal influences (Giraldo et al., 2013; Radtke et al., 2020). Such differences could affect the inflammatory and regenerative potential of PRP. Indeed, WBC-rich PRP has been shown to modulate tissue repair differently depending on WBC profile, suggesting that sex-specific responses may influence clinical outcomes (Boswell et al., 2013; Castillo Franz et al., 2021; Zhou et al., 2015).

By contrast, we found no significant sex-related difference in platelet concentration within PRP. This aligns with previous reports showing minimal or inconsistent influence of sex on equine PRP platelet counts (Geburek et al., 2016; Paz et al., 2022; Trevissón -Redondo et al., 2022). These findings reinforce the idea that, unlike leukocytes, platelet yields are less sensitive to sex-related intrinsic variation.

Broader intrinsic and extrinsic influences: Our results should also be interpreted within the broader context of factors influencing PRP composition. Age and training have been shown to significantly alter hematological parameters, with younger and actively trained horses exhibiting higher platelet and leukocyte counts (Giraldo et al., 2013; Paz et al., 2022; Valle et al., 2015). Extrinsic technical factors, including choice of anticoagulant and centrifugation methodology, can further modify PRP content and growth factor release (Fukuda et al., 2020; Textor et al., 2011). Such interactions highlight the multifactorial nature of PRP variability and underscore the challenges in achieving protocol standardization (Carr, 2021; Chen et al., 2017).

Limitations: This study has some limitations. The sample size was relatively modest (41 horses), and only two breeds were investigated, which may restrict the generalizability of the findings to the wider equine population. In addition, age and training status—both known to influence equine hematological parameters—were not stratified in this analysis. These factors could therefore have contributed to variability in the results and should be considered in future research designs.

Conclusion

This study demonstrates that both breed and sex influence the cellular composition of platelet-rich plasma in horses. TB horses yielded higher platelet concentrations in PRP than PA horses, while males exhibited higher WBC counts in PRP compared to females. In contrast, peripheral platelet counts and PRP platelet yields did not differ significantly by sex.

Taken together, these results show that intrinsic factors can partly explain the variability observed in equine PRP preparations. Recognizing breed- and sex-related differences is therefore important for clinicians when interpreting hematological data and optimizing regenerative protocols. Future research should involve larger cohorts and multiple breeds to refine standardization efforts and enhance the consistency of PRP-based therapies in equine medicine.

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